cells. Using surface plasmon resonance and centrifugation assays, we have found that the Smurf1 C2 domain binds to phosphoinositides and phosphatidylserine in an synergistic fashion. Confocal images of Smurf1 C2-GFP demonstrate that the domain localizes to the plasma membrane as well as intracellular vesicles in cells. Site-directed mutagenesis has shown the specific residues in the loop region of the protein involved in its cellular membrane localization. In addition, we have used a rapamycin-inducible phosphoinositide phosphatase system to demonstrate that this domain binds phosphoinositides at the plasma membrane. We conclude that the unique properties of the Smurf1 C2 domain to sense specific lipids in addition to anionic charge enable it to target multiple subcellular locations.

1539-Pos Board B269

Membranes in Flux: Proteins Effect on Membrane Permeability

Katie Dunleavy, Anika Rannikko, Anne Rice, Samantha Jaworski, Michael Fealey, Ryan Mahling, Anne Hinderliter.
Department of Chemistry and Biochemistry, University of Minnesota Duluth, Duluth, MN, USA.

Lipid membranes are the basis of cell structure by providing stability and regulation against certain stressors. The permeability of this membrane is regulated through the distribution of phospholipids across a lipid bilayer. With the addition of cholesterol to the lipid bilayer the mixing behaviors of the phospholipids present in the membranes are altered causing fluctuation in permeability. Cholesterol has the ability to move freely across monolayers, contributing to the communication passing through the bilayer. Annexin and the C2A domain of Synaptotagmin 1 are two types of membrane binding proteins whose binding affinity is affected in the presence of calcium ions (Ca^{2+}). With the combination of cholesterol containing lipid bilayers, Ca^{2+}, and protein the distribution of phospholipids and cholesterol is fluctuated, causing alteration in the membranes permeability. Both proteins were used in a carboxyfluorescein (CF) release assay to study the fluctuation in membrane permeability with increasing concentrations of protein. The measured efflux of this fluorescent dye in the presence of increasing concentrations of protein with and without Ca^{2+} shows these fluctuations in permeability across the lipid membrane. The amount of cholesterol present in the membrane correlates with the measured CF efflux released through the lipid bilayer upon binding of protein and Ca^{2+}. We hypothesize that membrane binding proteins have the ability to sense certain distributions of phospholipids and cholesterol across the lipid membrane and communicate changes within the membrane environment.

1540-Pos Board B270

Combinatorial Polarized TIRF-AFM Study of Membrane Reorganization by Alpha-Synuclein and Human Prion Protein

Martin Kurylowicz[1], Gillian Vanderlee[1], Daniel Ysselstein[2], Patrick Walsh[3], Jason Yau[3], Simon Sharpe[3], Jean-Christophe Rochet[2], Christopher M. Yip[4].
[1]Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, Canada, [2]Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN, USA, [3]Biochemistry, University of Toronto, Toronto, ON, Canada, [4]Chemical Engineering, University of Toronto, Toronto, ON, Canada.

Many neurodegenerative diseases are caused by aggregation or fibrillation of membrane-associated proteins. Alpha-Synuclein (aS) is associated with Parkinson's Disease as a major component of Lewy bodies, and misfolded aggregates of human Prion Protein (PrP) are associated with spongiform encephalopathies. Here we study the influence of these proteins on the organization and topography of canonical raft-mimetic supported lipid bilayers (DSPC/DOPC/cholesterol at 35/35/30 mol% on mica) using a correlated polarized Total Internal Reflectance Fluorescence-Atomic Force Microscopy (pTIRF-AFM) platform. pTIRF allows for high-speed measurements of dynamic processes at diffraction-limited spatial resolution, while AFM gives time-averaged nm-scale spatial resolution. Using pTIRF and an oriented lipophilic dye (DiI-C18) we determine the local order in the membrane by measuring the ratio of fluorescence parallel and perpendicular to the membrane plane, while simultaneously measuring membrane topography using AFM. Upon addition of aS to our phase-segregated membrane system, liquid-ordered and liquid-disordered domains were mixed and the local order increased such that lipid headgroups were oriented more parallel to the membrane plane. Moreover, AFM topography revealed the formation of long, closed-loop tubules. Addition of the active segment PrP(106-126) to these raft-mimetic membranes resulted in a similar mixing of domains and an increase of local order, while AFM revealed the formation of peptide fibrils in and around cholesterol-rich domains. This study provides direct evidence of membrane reorganization and restructuring by aS and PrP, and the effect of membrane chemistry on aS and PrP self-association. These results portend future detailed investigations into the molecular-level factors responsible for these effects.

1541-Pos Board B271

Superresolution Plasmalemmal Lipid Mapping

Brian L. Ross, Jin Zhang.
Biomedical Engineering, Johns Hopkins University, Baltimore, MD, USA.

Charged lipid species in the plasma-membrane play an integral role in a variety of signaling pathways, in which they function as second messangers as well as docking sites for proteins. It has been suggested that the spatial organization of charged lipids called phosphoinositides is not random_rather, they form nanoscale clusters that are distinct from lipid rafts. Through super-resolution imaging in live cells, we are investigating the role of these phosphoinositide nanodomains in providing spatial regulation of the PI3K/Akt and IP3 signaling pathways. Using PALM and photochromatic SOFI (pcSOFI) superresolution techniques, we are working to create high resolution, time-resolved lipid maps of the inner-leaflet of the plasma membrane. Lipids are probed using various fluorescent protein-tagged pleckstrin homology domains, which are coimaged with each other, with lipid raft markers, and with protein subunits. The resulting lipid maps will provide information on the how lipids in the plasma membrane are organized, and how this organization helps provide spatial regulation of the PI3K/Akt as well as the IP3 signaling pathways.

1542-Pos Board B272

Spatial Organization of Oncogenic Ras on Supported Membranes

Hiu Yue Monatrice Lam, Jean K. Chung, Wan-Chen Lin, Jay T. Groves.
Chemistry, UC Berkeley, Berkeley, CA, USA.

Ras is a membrane protein which acts as a molecular switch in the activation of many signal transduction pathways involved in cellular processes such as proliferation and apoptosis. Ras has been a subject of great interest due to the causal role found between its hyperactivity and cancer, and studies have shown that approximately 30% of all human cancers have oncogenic Ras mutations. Although Ras has been studied for more than thirty years, the spatial organization of oncogenic Ras on membranes is still not well characterized. It has been suggested that Ras may organize into clusters in cell membranes but the molecular scale structure of these organizations remain unknown. Molecular dimerization of Ras has also been suggested and we quantitatively characterize this in an array of Ras mutants in supported lipid bilayer membranes using fluorescence correlation spectroscopy (FCS) and photon counting histogram analysis (PCH). The implications of Ras clustering on downstream signaling will be discussed.

1543-Pos Board B273

The Hidden Role of Residues in the Recognition Center of Human Glycolipid Transfer Protein (GLTP)

Borja Ochoa-Lizarralde[1], Sandra Delgado[1], Xiuhong Zhai[2], Valeria R. Samygina[1], Alexander N. Popov[3], Aintzane Cabo-Bilbao[1], Helen M. Pike[2], Julian G. Molotkovsky[4], Dinshaw J. Patel[5], Rhoderick E. Brown[2], **Lucy Malinina**[1].
[1]CIC bioGUNE, Technology Park de Bizkaia, Derio, Spain, [2]Hormel Institute, University of Minnesota, Austin, MN, USA, [3]European Synchrotron Radiation Facility, Grenoble, France, [4]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russian Federation, [5]Structural Biology, Memorial Sloan-Kettering Cancer Center, New York, NY, USA.

Human glycolipid transfer protein (hsGLTP) represents the prototypical GLTP-fold, characterized by a sandwich topology that forms a hydrophobic pocket for encapsulating the ceramide moiety. The glycolipid-headgroup recognition center located at the protein surface provides a hydrogen bond network and hydrophobic contacts to specifically recognize the sugar-amide region of the glycolipid. On the other hand, the recognition center also includes residues whose role in glycolipid recognition is unclear. Herein, we investigated the role of such residues by introducing multiple mutations combined with analyses of the signature response in intrinsic tryptophan fluorescence of GLTP (Zhai et al., 2009, J Biol Chem. 284, 13620-13628) due to ligand binding. The approach enables discrimination of normal ''high-affinity'' complexes from low-affinity or super-high-affinity ones. Glycolipid transfer assays provide insights into how the mutations affect intermembrane transfer activity and glycolipid selectivity. Data show that Lys55 keeps the affinity for galactosyl¬ceramide similar to other glycolipids, such as glucosylceramide or sulfatide; whereas Leu92 and the C-terminal Val209 residue adjust the affinity for all the three glycolipids (sulfatide, glucosyl- and galactosylceramide) to optimize their binding for efficient intermembrane transfer. Crystal structures of select super-high- and low-affinity complexes provide insights into intermolecular interactions and protein